ELECTROCHEMICAL STUDY OF MANNITOL OXIDATION AT NICKEL OXIDE ELECTRODE

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Dedicated to Professor Sergio Roffia on the occasion of his retirement.

The electrocatalytic oxidation of mannitol at a nickel oxide electrode was investigated. The experimental conditions for determining mannitol concentrations have been optimised taking into account the involved electrochemistry. Unlike what previously reported in the literature, our findings lead to the conclusion that both the electrochemical reaction involving the Ni(II)/Ni(III) couple and the chemical reaction between mannitol and Ni(III) are effective in determining the overall kinetics of the electrocatalytic process. The calibration line for mannitol was linear up to 20.0 mmol l^{-1}. Mannitol determination with the nickel oxide electrode was performed in a liquid culture medium selective for *Staphylococcus aureus* in order to make an indirect calibration of bacterial viable cells, but the results were not satisfactory.

Keywords: Electrochemistry; Nickel oxide electrode; Sensors; Electrocatalysis; Mannitol determination; Bacteria; Alditols; Carbohydrates; Oxidations.

In the field of food chemistry the determination of carbohydrates and their degradation products is of great interest and many assays, based on potentiometric and/or amperometric sensors, have been used for this purpose[1,2]. Many articles report the use of platinum, gold, copper, cobalt, nickel materials[2–7], and phthalocyanine-modified carbon paste electrodes[8] often employed as electrochemical detectors in liquid chromatography and flow-injection analysis. In most of these cases, the electrode process, which usually occurs in strongly alkaline solution, involves solute oxidation at a metal oxide or hydroxide surface. The major drawback of Pt or Au is fouling

of the electrode due to reactant/product adsorption effects, which can be overcome by a continuous pulsing of the surface to extreme positive and/or negative potentials.

Nickel has been demonstrated to be a good candidate for the development of a carbohydrate sensor. For Ni, the key step is the electrochemical production of a Ni(III) surface state, usually described as NiOOH, which functions as the redox mediator between substrate and electrode[1,5–7,9], thus allowing a simple, constant-potential amperometric detection.

From the food safety view, the majority of tests carried out by the agricultural/food industry is aimed at checking the presence of micro-organisms (*e.g.*, pathogens and spoilage organisms), microbial toxins (*e.g.*, mycotoxins and bacterial toxins), antibiotic residues, pesticides, and synthetic hormones. Estimation of microbial load is very important for the control of food hygiene, but conventional detection procedures need more than 24–48 h to obtain results, a time that is incompatible with online quality control. There is a widespread need of analytical methods for the rapid and inexpensive detection of microbial contamination. The state of the art of such detection techniques has been reviewed by Hobson *et al.*[10]

In this paper we report the results of a study carried out to elucidate the electrochemistry involved in the catalytic oxidation process of mannitol at a nickel oxide electrode in order to optimise the experimental conditions.

Mannitol has been chosen since it could be used for indirect calibration of viable cells of *Staphylococcus aureus* grown in a highly selective medium, where mannitol is the fermentable saccharide.

The growth of *S. aureus* in foods presents a potential public health hazard since many strains produce enterotoxins that cause food poisoning if ingested. Raw milk and unpasteurized dairy products may contain large numbers of *S. aureus*, usually as a result of staphylococcal mastitis in the cattle breeding[11]. An early discovery of the infection is very important from the economic point of view.

EXPERIMENTAL

Electrochemical Instrumentation

All electrochemical experiments were carried out using a CH Instruments 620-electrochemical workstation driven by its software. A conventional three-electrode cell was used under nitrogen atmosphere. A platinum grid was used as a counter electrode. All potentials were referred to SCE. The working electrode consisted of a nickel wire (1 mm in diameter, 99.99%, Aldrich) in epoxy resin (Buehler) in order to expose only a nickel disk (apparent

area 0.03 cm^2) to the solution. Electrode surface was mirror-polished with fine alumina powder (0.05 μm).

Chemicals and Reagents

Sodium hydroxide, sodium chloride, sodium L-lactate, sodium pyruvate, and D-mannitol were of analytical reagent grade and were purchased from Fluka. All chemicals were used as received. All solutions were prepared with water doubly distilled from a glass apparatus.

Electrochemical Measurements

Calibration plots for mannitol, pyruvate and lactate were constructed from the cyclic voltammograms (CVs) recorded at 50 mV s^{-1} in 0.5 M NaOH, containing different concentrations of the analytes. When analysing the filtered trypticase soy broths (TSB), the CVs were recorded at the same scan rate on solutions obtained by adding 5.5 ml of aqueous 2 M NaOH to 1.5 ml of the broth.

Microbiological Tests

Cultures of *S. aureus* (ATCC 25923) grown on TSB broth (Difco) with 1% of Na-pyruvate at 35 °C for 24 h were diluted $1:10^5$ and $1:10^6$ and from these predilutions further dilutions were obtained and inoculated in two series of trypticase soy broth containing 10% NaCl, 1% sodium pyruvate and 0.25% mannitol. The tubes were incubated at 35 °C for 8 h. The broths were filtered through 0.22 μm sterile filters (Millex-GS, Millipore) and filtrates collected in sterile tubes which were immediately frozen at –18 °C until the electrochemical experiments were performed. Viable counts in the predilutions were calculated by the most probable number (MPN) method described in the procedure 33.52 of ref.[11]

RESULTS AND DISCUSSION

Nickel Oxide Electrode Preparation

The nickel oxide electrode (NOE) system has been the object of extensive study as to both electrochemical and structural aspects. Since 1960 many articles have been devoted to the passivation phenomena and to the study of composition of oxidation films[12]. In 1960 Bode *et al.*[13] demonstrated the electrochemical formation of at least two kinds of nickel hydroxides, α- and β-$Ni(OH)_2$, and two kinds of nickel oxide hydroxides, β- and γ-NiOOH, which differ by their electrochemical properties and by their crystalline structure. The original scheme of Bode was further developed by Arvia *et al.*[14], who showed the existence of another hydroxide form, denoted $Ni(OH)_2$*. Nickel metal in contact with an alkaline solution is spontaneously covered with a thick layer of α-$Ni(OH)_2$ [5,15]. Electrochemical ageing of α-$Ni(OH)_2$ gives slowly, but irreversibly β-$Ni(OH)_2$, which is a thermodynamically stable species. For this reason the fresh electrodes have to be pre-

conditioned to improve the conversion rate in order to obtain a reproducible signal. Figure 1 shows several cyclic voltammograms recorded for NOE in 0.5 M NaOH, in the potential range from 0.000 to 0.600 V. In the anodic region, the conversion of α-$Ni(OH)_2$ to β-$Ni(OH)_2$ is observed as the number of cycles increases (peak A→B). E_{pa} shifts progressively from 0.380 to 0.430 V. The one-electrode process

$$Ni(OH)_2 + OH^- \rightarrow NiO(OH) + H_2O + e^- \qquad (1)$$

gives β- and γ-NiO(OH), which explain the existence of two reduction peaks, C and D, during the negative sweep. In the cathodic part we observe a progressive increase in the peak associated with the reduction of γ-NiO(OH) (peak D), which was hardly noticeable during the first cycles. Starting from the 1000th cycle, the steady state of the oxidation peak was reached.

We tried also another method of preparation the nickel oxide electrode, *i.e.*, potentiostatically. The β-$Ni(OH)_2$ phase was formed on the electrode surface by applying a potential of 0.500 V in 0.5 M NaOH for 3600 s. The electrochemical signal of such an electrode was not as reproducible as the one obtained potentiodynamically and we had to apply about 300 potential cycles, from 0.000 to 0.600 V, in order to obtain a steady-state response. Similar results could be obtained by ageing the electrode for 12 h in the

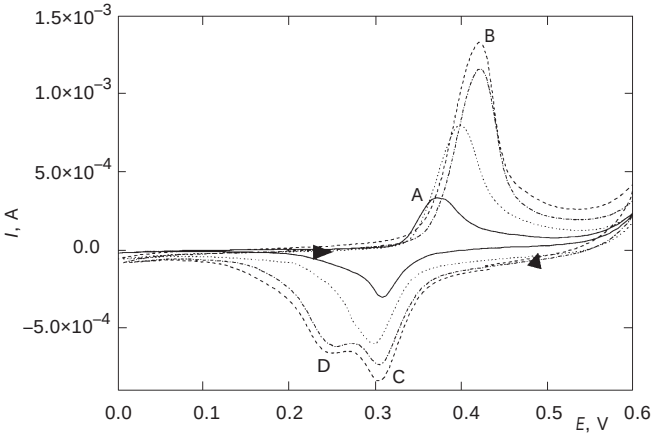

FIG. 1
Cyclic voltammograms for Ni oxidation in 0.5 M NaOH. Scan rate: 50 mV s^{-1}. —— 10° cycle, · · · · · 200° cycle, – · – · – 400° cycle, - - - - - 900° cycle

same electrolytic solution. These findings are in agreement with the literature[6,10] even if we have used a more alkaline solution, which permits a better peak separation from the solvent anodic limit[10].

All the electrodes used in this work for the study of the electrocatalytic mechanism were prepared by the potentiodynamic procedure. The stability of such an electrode was tested by recording cyclic voltammograms within a time period of 12 days. The current responses changed no more than 3%.

Cyclic voltammograms recorded at various scan rates show a linear dependence of I_p *vs* v, up to 10 mV s^{-1}, *i.e.*, a thin layer behaviour, characteristic of a modified electrode for which the electrochemical reaction is surface-confined. The observed separations between anodic and cathodic peaks markedly deviated from a surface-confined electrode process with fast electrode transfer behaviour[16]. From the evaluation of the charge associated with the oxidation process in the thin layer regime, we estimated a thickness of a few tenths of oxide layers involved in the redox process. On the contrary, at higher scan rates, we obtain a dependence on $v^{1/2}$, *i.e.*, the electrode process is diffusion-controlled.

Electrocatalytic Properties

NOE has been widely employed for the electrocatalytic oxidation of a wide class of organic compounds, *e.g.* alcohols, carbohydrates, amines and amino acids[1,5–7,9].

Fleischmann *et al.*[6] reported that alcohols and amines are oxidised at nickel anode in aqueous solutions. They showed that the rate-determining step is the chemical oxidation of the substrate with NiO(OH) formed anodically on the electrode surface.

$$\mathrm{NiO(OH)} + \text{analyte (adsorbed)} \xrightarrow{\text{Rate determining step}} \mathrm{Ni(OH)_2} + \text{radical} \qquad (2)$$

$$\text{radical} \rightarrow \text{products} \qquad (3)$$

We investigated the electrocatalytic response of NOE to mannitol. Figure 2 shows the cyclic voltammograms obtained in mannitol-free 0.5 M NaOH solution and at increasing mannitol concentrations.

Several features of these CVs must be discussed in order to clarify the involved electrochemistry:

1. the anodic peak current (I_{pa}) increases as the mannitol concentration increases;

2. all the CVs show, in the reverse scan, a residual reduction wave, whose intensity decreases as the oxidisable substrate concentration increases;

3. the E_{pa} shifts towards higher values as the mannitol concentration increases;

4. in the reverse scan, it is possible to observe an anodic (catalytic) current persisting at lower and lower potential values as the mannitol concentration increases (curve *3*). From a concentration value upward (curve *4*), the curve in the reverse scan crosses the forward one (hysteresis).

The increment of the anodic peak is proportional to mannitol concentration and clearly demonstrates the electrocatalytic properties of NOE. The peak-shaped curves indicate an electrocatalytic process limited by the chemical reaction of the substrate with catalytic nickel sites in the concentration range explored (see below).

Moreover, all the CVs show, in the reverse scan, a residual reduction wave and a shift of E_{pa} towards higher values as the mannitol concentration increases. The shift is remarkable at low mannitol concentrations (25 mV/mmol l^{-1}) and becomes almost negligible (2.5 mV/mmol l^{-1}) at mannitol concentrations greater than 5 mmol l^{-1}. The effect of increasing mannitol concentration leads to a catalytic reaction that can be sustained at more anodic potentials due to higher Ni(II)/Ni(III) ratio resulting from a greater amount of substrate available inside the NOE structure. The involve-

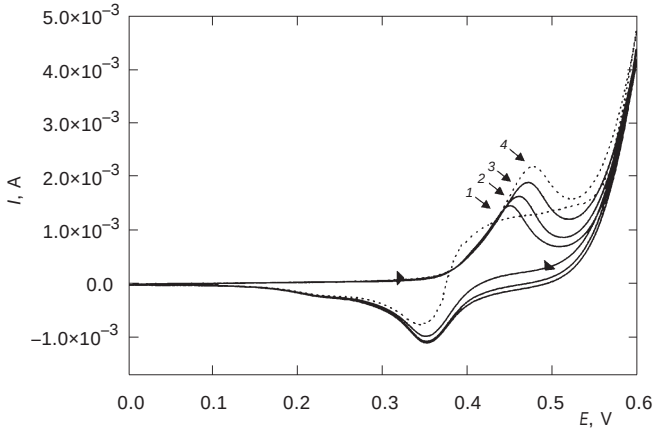

FIG. 2

Cyclic voltammograms recorded at NOE in 0.5 M NaOH, without (*1*) and with addition of mannitol at concentrations 1.8 (*2*), 5.7 (*3*), and 10.0 mmol l^{-1} (*4*). Scan rate: 50 mV s^{-1}

ment of more nickel sites in the catalytic process decreases the number of Ni(III) sites that are reduced in the subsequent cathodic scan[17].

These phenomena can be partly understood taking into account the structure characteristics of the species involved in the electrochemistry of NOE. The compound β-$Ni(OH)_2$ can be described as a layered structure consisting of a hexagonal planar arrangement of Ni(II) ions with an octahedral co-ordination of oxygen lying out of the plane[15]. The layers are stacked up along the *c* axis. This layered structure permits intercalation of the oxidisable substrate since the steric hindrance of mannitol is compatible with the interlayer distance.

On the basis of this structure we have to consider for NOE two kinds of diffusion-limiting processes: the diffusion of the substrate from the bulk of solution to the nickel oxide electrode interface and the diffusion inside the layers. The two distinct processes take place at different time scales, due to different values of the diffusion coefficients. The relevant time domain could be explored, in principle, by following the performance of the electrocatalytic process, *i.e.* the I_{cat}, at different scan rates. I_{cat} has been evaluated as the difference between the I_{pa} measured in presence of the substrate and the I_{pa} measured in a mannitol-free solution I_{noncat}.

Figures 3a and 3b show the dependence of I_{cat} *vs* the square root of the scan rate, and *vs* the scan rate, respectively. The CVs have been recorded in a solution, which was 7.0 mM in mannitol and 0.5 M in NaOH, with the scan rate ranging between 5 and 750 mV s^{-1}. The I_{cat} appears linearly correlated with the square root of the scan rate indicating a process controlled by diffusion of the electroactive species, *i.e.*, the oxidisable substrate, to Ni

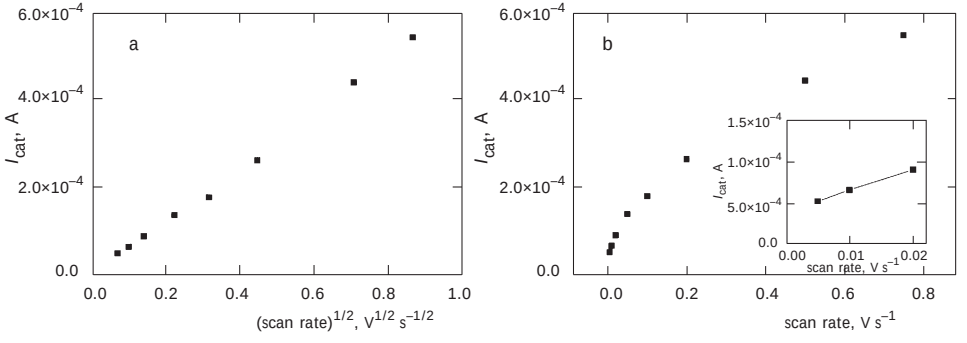

FIG. 3

Plots of I_{cat} *vs* the square root of potential scan rate (a) and scan rate (b) obtained for a 7.0 mM mannitol solution in 0.5 M NaOH. Inset: the same plot relative to low potential scan rates

active sites of NOE. A close inspection (see inset in Figure 3b) reveals a linear dependence on the scan rate at values lower than 20 mV s^{-1}. This value probably establishes the time scale at which it becomes important to consider the diffusion inside the layered structure of NOE. In both cases, the available oxidisable substrate is always deficient with respect to the catalytic sites. For this reason, the electrocatalytic process never reaches the steady state. This particular behaviour is confirmed by the fact that the reduction peak in the cathodic region is always present at all the investigated scan rates.

Casella *et al.*[6] reported a detailed study of the electrocatalytic oxidation at NOE of mono- and polyhydric alcohols. They proposed two reaction schemes, in which the rate-determining step was the chemical reaction of the substrate, previously adsorbed on the oxidised surface of the electrode. Our results are in agreement with these findings. In particular, the CVs recorded at high mannitol concentrations are affected by strong hysteresis (Fig. 2) with catalytic currents, in the reverse scan, higher than those observed in the forward scan. This cyclic-voltammogram morphology is consistent with an electrocatalytic process in which the rate of the chemical reaction is lower than that of the electrochemical reaction[17]. In order to give a deeper insight on the mechanism of mannitol electrooxidation, our experimental results have been elaborated taking into account the electrocatalytic efficiency (EE), defined as the current ratio I_{cat}/I_{noncat}. Figure 4 reports a bar diagram of EE at various scan rates and different mannitol

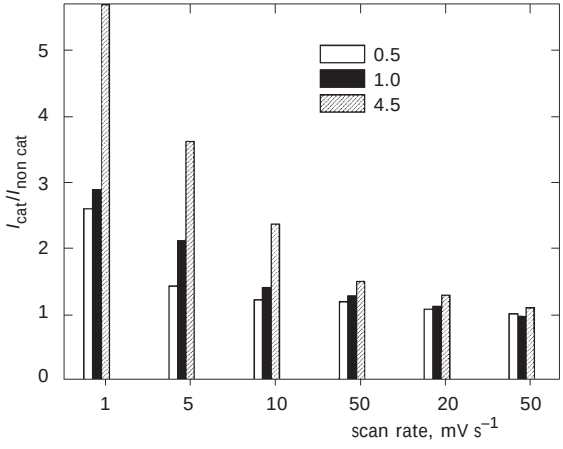

FIG. 4

Bar diagram of the electrocatalytic efficiency of NOE towards mannitol (at the concentrations shown in the frame) *vs* potential scan rate

concentrations. The electrocatalytic efficiency is greater at low scan rates and at higher substrate concentrations. The peculiar dependence of the EE on the scan rate is in accordance with the performance of a catalytic system where the rate of the electrochemical reaction (reaction (*1*)) competes with the rate of the chemical reduction, regeneration reaction (*2*)[18]. When the scan rate is lowered, more substrate can be oxidised, whereas at high scan rates the amount of the oxidised substrate decreases, *i.e.*, the catalytic current is smaller.

As a consequence, our findings lead to the conclusion that both reactions (*1*) and (*2*) are effective in determining the overall reaction kinetics.

Analytical Applications

Once the experimental conditions to obtain the best electrocatalytic response of NOE to mannitol were established, we made calibration graphs by plotting the I_{cat} obtained from CVs recorded at 50 mV s^{-1} in 0.5 M NaOH *vs* mannitol concentration. After each addition of substrate, the electrode response in the presence of only sodium hydroxide was recorded in order to verify the reproducibility of the blank signal, *i.e.* I_{noncat}, which was satisfactory (the coefficient of variation was less than 2.1% for $n = 5$). The calibration graphs were constructed from ten additions of mannitol in the concentration range up to 20.0 mmol l^{-1}. A least-squares regression fit showed good linearity ($R^2 = 0.9991$) with a slope value of 6.56 ± 0.13 μA/mmol l^{-1}.

In order to check if mannitol determination could be related to bacterial viable cells counts, the calibration line for mannitol was obtained also in the presence of trypticase soy broth, *i.e.*, under the same conditions utilised for the estimation of *S. aureus* loads by the classical MPN counting technique. A linear relationship was again obtained between mannitol concentration and current response up to 3.5 mmol l^{-1}, corresponding to the maximum level of the substrate fermentable by *S. aureus*, after dilution of the broth (1.5 ml) with 2 M NaOH (5.5 ml), which is found when the bacterium is absent. In such a case the value of the slope of the calibration line decreased by about a factor of 4 with respect to the value obtained in the presence of only NaOH.

Since the effect of the various components present in the growth medium led to a different response of the sensor, but linearity was still observed, we used this regression line to evaluate the correlation for the data obtained by MPN method to enumerate viable cells of *S. aureus*, and the data from the electrocatalytic response of NOE to estimate mannitol concentration. In principle, such a correlation was expected to be linear with a

correlation coefficient of –1. Actually, the resulting correlation was not acceptable (R = –0.4).

Since NOE is also electroactive towards lactate, we assumed the result could be ascribable to the presence in the nutrient medium of this substance, which is the prevailing product from the homolactic fermentation of *S. aureus*. The calibration line obtained for lactate in 0.5 M NaOH showed a slope about one order of magnitude smaller than that observed for mannitol.

This result, even if it explained the positive interference of lactate amplified by the fact that the lactate concentration increases as mannitol is fermented, should not increase the current peak enough to make the correlation MPN *vs* mannitol concentration so poor. In fact, the range of bacterial loads taken into account was low, from 2.4 to 2.4 × 10^3 MPN ml^{-1}.

CONCLUSIONS

We have investigated by cyclic voltammogram the electrocatalytic oxidation of mannitol at the nickel oxide electrode. Our results suggest, unlike what has been previously reported in the literature, that both the chemical reaction between the substrate and the NiO(OH) formed on the electrode surface and the electrochemical reaction involving the Ni(II)/Ni(III) couple are effective in determining the overall kinetics. This finding clarifies better the mechanism of the electrocatalytic reaction of mannitol at NOE. The mannitol determination has been carried out in 0.5 M NaOH, observing calibration linearity up to 20.0 mmol l^{-1}. The measurement of mannitol concentration has been performed in a growth medium selective for *S. aureus* in order to verify a correlation with the bacterial viable cell counts. Unfortunately, the obtained correlation was poor due to the interferences deriving from the other components of the growth medium.

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